Abstract only

1: Cancer Res 1993 Sep 1;53(17):3860-4

Use of tissue-specific expression of the herpes simplex virus thymidine kinase gene to inhibit growth of established murine melanomas following direct intratumoral injection of DNA.

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We report here the use of the 5' flanking region of the murine tyrosinase gene to direct expression of the herpes simplex virus thymidine kinase (tk) gene specifically to murine melanoma cells, whilst not permitting expression in a range of other cell types. Expression of the herpes simplex virus tk gene from the tyrosinase promoter in melanoma cells rendered them sensitive to killing by ganciclovir (100% cell death of a tk-expressing B16 clone after 12 days in culture at 1 microgram/ml ganciclovir). We also observed a substantial bystander killing effect when expressing cells were mixed with nontransfected parental B16 cells. When transfected murine melanoma cells expressing tk were injected into syngeneic mice both their tumorigenicity and experimental metastatic potential were abrogated completely when the mice were treated with ganciclovir (27 of 28 mice treated with water developed progressively growing tumors versus 1 of 30 in the ganciclovir-treated group). Direct injection of the tk gene under control of the tyrosinase promoter into established tumors in mice, followed by treatment with ganciclovir, led to significant reductions in resultant tumor size relative to the size of tumor developing in mice treated with water (median tumor weight, 1.65 g versus 2.75 g). Therefore, direct transfer of recombinant genes by injection of DNA can significantly reduce established tumor burden in vivo.

PMID: 8395331 [PubMed - indexed for MEDLINE]

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Abstract only

☐ 1: Cancer Res 1993 Mar 1;53(5):962-7

In vitro and in vivo targeting of gene expression to melanoma cells.

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Gene therapy protocols for cancer usually involve removal of tumor cells, culture in vitro to allow gene transfer, and subsequent reintroduction in vivo. Targeting therapeutic genes to tumor cells in situ requires an accuracy of gene delivery that currently is not possible with the use of existing techniques. To overcome these limitations we have used two promoters, which are preferentially active in melanocytic cells, to direct gene expression specifically to melanoma cells both in vitro and in vivo. Here we describe experiments showing that as little as 769 base pairs of the 5'-flanking regions of the tyrosinase, and 1.4 kilobase pair of the tyrosinase-related protein 1, genes are sufficient to direct expression of the beta-galactosidase gene to both human and murine melanoma cells and melanocytes, while not permitting expression in a range of other cell types in vitro. These promoters showed high levels of activity in 12 of 14 murine and human melanoma cell lines tested but showed only basal levels of activity, similar to that of a promoterless construct, in a range of 12 other cell types. Cell type specificity is maintained when the construct is delivered to cells either by physical means or by inclusion of the cell type-specific expression cassette into a retroviral vector. Direct injection of DNA, encoding the beta-galactosidase gene expressed from either promoter, into established B16 melanomas or Colo 26 tumors in syngeneic mice resulted in extensive transduction of tumor cells in the B16 melanomas (approximately 10% of tumor cells expressing 10 days after DNA injection), whereas no blue-staining cells were seen in the Colo 26 tumors. The reporter gene was expressed in melanoma cells and in some normal melanocytes but not in other surrounding normal tissue. We propose that the combination of a tissue-specific promoter driving a therapeutic gene, with delivery of such a construct directly to sites of tumor growth in vivo, either by direct DNA injection or by retroviral infection, may provide significantly enhanced safety for gene therapy for solid tumors.

Abstract only

☐ 1: J Biol Chem 1987 Apr 5;262 (10):4812-8

Cell-specific enhancer activity in a far upstream region of the human alpha-fetoprotein gene.

Watanabe K, Saito A, Tamaoki T.

We describe experiments showing that the 5'-flanking region of the human alpha-fetoprotein (AFP) gene contains transcription control elements with characteristics of enhancers. The enhancer activity was detected and characterized by the ability to direct the expression of a linked chloramphenicol acetyltransferase (CAT) gene in transfected AFP-producing hepatoma cells in culture. The enhancer activity is cell-specific in that it occurs in hepatoma cells producing AFP, but not in non-AFP-producing hepatoma or nonhepatic cells. The active elements can direct CAT expression in conjunction with the SV40 promoter in an orientation- and position-independent manner. The sequences important for enhancer activity reside in the 400-base pair region between 3.3 and 3.7 kilobase pairs upstream of the AFP gene. This and proximal upstream regions contain multiple enhancer "core"-like sequences and other stretches of potential biological significance.

PMID: 2435718 [PubMed - indexed for MEDLINE]

☐ 1: Mol Endocrinol 1991 Dec;5 (12):1921-30

The promoter of the prostate-specific antigen gene contains a functional androgen responsive element.

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Expression of prostate-specific antigen (PA) mRNA was tested at various time periods after incubation of the human prostate tumor cell line LNCaP with the synthetic androgen R1881. Androgen-stimulated expression was observed within 6 h after addition of R1881 to the cells. Run-on experiments with nuclei isolated from LNCaP cells showed that expression of the PA gene could be regulated by R1881 on the level of transcription. DNase I footprints of the promoter region of the PA gene (-320 to +12) with nuclear protein extracts from LNCaP cells showed at least four protected regions. The protected areas include the TATA-box, a GC-box sequence, and a sequence AGAACAgcaAGTGCT at position -170 to -156, which closely resembles the reverse complement of the consensus sequence GGTACAnnnTGTTCT for binding of the glucocorticoid receptor and the progesterone receptor. Fragments of the PA promoter region were cloned in front of the chloramphenicol acetyltransferase (CAT) reporter gene and cotransfected with an androgen receptor expression plasmid into COS cells in a transient expression assay. CAT activity of COS cells grown in the presence of 1 nM R1881 was compared to untreated controls. A 110-fold induction of CAT activity was found if a -1600 to +12 PA promoter fragment was used in the construct. By further deletion mapping of the PA promoter a minimal region (-320 to -155) was identified as being essential for androgen-regulated gene expression. Mutation of the sequence AGAACAgcaAGTGCT (at -170 to -156) to AAAAAAgcaAGTGCT almost completely abolished androgen inducibility of the reporter gene constructs. One or more copies of the sequence AGAACAgcaAGTGCT cloned in front of a thymidine kinase promoter-CAT reporter gene confers androgen regulation to the reporter gene. These findings provide strong evidence for transcription regulation of the PA gene by androgens via the sequence AGAACAgcaAGTGCT. Interestingly, in addition to the AGAACAgcaAGTGCT element, an upstream region (-539 to -320) is

needed for optimal androgen inducibility of the PA promoter.

PMID: 1724287 [PubMed - indexed for MEDLINE]
